the MLC 1/3 enhancer sequence was inserted into the construct. The gene construct was then inserted into a rAAV transfer plasmid (pSUB201) between the inverted terminal repeat sequences (ITRs) of the virus.

Figure 1B, comprising three panels, is an image of a gel depicting the persistence of IGF-I expression in muscle following injection therein with rAAV- IGF-I wherein IGF-I expression was detected by reverse-transcriptase polymerase chain reaction (RT-PCR) assay. The lanes were loaded with sample RT-PCR reactions performed on nucleic acid obtained from the following muscle tissues: Lane 1, 27-month-old extensor digitorum longus (EDL) 9 months post-rAAV-IGF-I injection; lane 2, 6-month-old EDL at 4 months post-rAAV-IGF-I injection; lane 3, uninjected age-matched control EDL; lane 4, positive control using the rAA-IGF-I gene construct depicted in Figure 1A. The expression of IGF-I in the various muscle tissues compared to the positive control in lane 4 is depicted in the top panel of Figure 1B. The constitutive expression of β-cytoplasmic actin in EDL is depicted in the middle panel of Figure 1B. In this panel, lanes 1-3 were loaded as described previously. Lane 4 is a positive control using a template supplied with oligonucleotides obtained from Clontech Laboratories, Inc. (Palo Alto, CA). In the bottom panel of Figure 1B, data demonstrating the integrity of RNA isolated from EDL muscles which was analyzed as described in the top and middle panels is shown. Equal volume RNA samples were electrophoresed through a non-denaturing agarose gel (Invitrogen, Carlsbad, CA). Lanes 1-3 were loaded as described previously herein.

Figure 2A , comprising two panels, is an image of photomicrographs depicting the effect of IGF-I expression in EDL muscles of young (6 month old) mice 4 months post-rAAV-IGF-I injection. Cross-sections of control non-injected and rAAV-IGF-I-injected EDL muscles were stained with hemotoxilin and eosin. rAAV-IGF-I-injected EDL ("Injected" panel) displayed significant increase in cross-sectional area compared with contralateral non-injected EDL ("Control" panel). Muscle fiber regeneration was evident in rAAV-IGF-I-injected EDL as demonstrated by the presence of central nuclei (insert, "Injected" panel) which were absent in non-injected EDL muscle (insert, "Control" panel). Scale bars equal 100 μm.

Figure 2B is a graph depicting the effect of IGF-I expression on muscle mass and force generation. Muscle mass (wet weight) and isometric tetanic force of rAAV-IGF-I-injected muscles were expressed relative to the same measurements in the contralateral non-injected muscles. The symbol "*" denotes $p<0.05$ for paired comparisons between rAAV-IGF-I-injected and control muscles.

Figure 3, comprising three panels, is a series of graphs depicting the effect of IGF-I expression in EDL muscles of old (27-month) mice compared to control non-injected young (6-month) mice and old (27-month) mice EDLs. Left panel: This graph depicts (from left to right) the muscle mass (wet weight) of young, control EDL; old, control EDL; and rAAV-IGF-injected old EDL, respectively. Middle panel: This graph depicts (from left to right) the tetanic force of young control EDL, old control EDL, and rAAV-IGF-I-injected old EDL, respectively. Right panel: This graph depicts (from left to right) the specific force of young control EDL, old control EDL, and rAAV-IGF-I-injected old EDL, respectively. For determination of specific force, the cross-sectional area of the EDL muscle was estimated using muscle mass and optimum muscle length pursuant to Brooks and Faulkner (1988, J. Physiol. 404:71-82). The symbol "*" denotes $p<0.05$ for comparisons to young adult control muscles.

Figure 4 is the nucleotide sequence of the rat liver form of IGF-1 (SEQ ID NO:3).

Figure 5 is an image of the muscles of a transgenic mouse having the MLC/IGF-1 construct, compared with the muscles of an otherwise identical non-transgenic mouse.

Remarks

The Examiner has objected to the specification pursuant to 37 CFR §§ 1.821-1.825. Specifically, the Examiner objects to the specification in that sequences that are disclosed in the specification are not identified by their sequence identifiers, especially in Figure 4 and the description thereof. Applicants have amended the specification, specifically the section entitled Brief Description of the Drawings, to include the sequence identifiers. As detailed in the specification at page 25, line 2, the

Clean Copy of the Brief Description of the Drawings

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a diagram depicting the structure of the IGF-I rAAV construct. Rat IGF-I cDNA was placed under the control of a fast muscle-specific promoter/regulatory sequence (myosin light chain 1/3 termed "MLC 1/3") positioned at the 5' end of the IGF-I cDNA. A SV40 polyadenylation sequence (SV40pA) was positioned at the 3' end of the IGF-I cDNA. On the 3' end of the SV40pA sequence, the MLC 1/3 enhancer sequence was inserted into the construct. The gene construct was then inserted into a rAAV transfer plasmid (pSUB201) between the inverted terminal repeat sequences (ITRs) of the virus.

Figure 1B, comprising three panels, is an image of a gel depicting the persistence of IGF-I expression in muscle following injection therein with rAAV- IGF-I wherein IGF-I expression was detected by reverse-transcriptase polymerase chain reaction (RT-PCR) assay. The lanes were loaded with sample RT-PCR reactions performed on nucleic acid obtained from the following muscle tissues: Lane 1, 27-month-old extensor digitorum longus (EDL) 9 months post-rAAV-IGF-I injection; lane 2, 6-month-old EDL at 4 months post-rAAV-IGF-I injection; lane 3, uninjected age-matched control EDL; lane 4, positive control using the rAA-IGF-I gene construct depicted in Figure 1A. The expression of IGF-I in the various muscle tissues compared to the positive control in lane 4 is depicted in the top panel of Figure 1B. The constitutive expression of β-cytoplasmic actin in EDL is depicted in the middle panel of Figure 1B. In this panel, lanes 1-3 were loaded as described previously. Lane 4 is a positive control using a template supplied with oligonucleotides obtained from Clontech Laboratories, Inc. (Palo Alto, CA). In the bottom panel of Figure 1B, data demonstrating the integrity of RNA isolated from EDL muscles which was analyzed as described in the top and middle panels is shown. Equal volume RNA samples were electrophoresed through a non-denaturing agarose gel (Invitrogen, Carlsbad, CA). Lanes 1-3 were loaded as described previously herein.

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B1 Cont

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